

**ABSTRACT:**

The objective of the current study was to establish an efficient and reproducible in vitro plant regeneration protocol using cotyledonary explant for *Citrullus lanatus* cv. Round Dragon. To achieve optimal conditions for adventitious shoot induction, cotyledon explants of 5-day-old seedlings, 7-day-old seedlings and 9-day-old seedlings were tested for regeneration potential on Murashige and Skoog (MS) media supplemented with 2.3 mg L<sup>-1</sup> BAP. Results showed that high frequency of in vitro adventitious shoot regeneration was induced from the proximal region of 5-day-old seedlings (93%) with 19.80±0.99 shoots per responding explant after 6 weeks. Adventitious shoots induced from 5-day-old seedlings after 6 weeks were transferred to MS shoot regeneration medium without plant growth regulator for shoot elongation for 4 weeks. The influence of various concentrations of IBA, IAA and NAA on root initiation was examined on half-strength and full-strength of MS rooting medium. The best response for root initiation was obtained from the microshoots grown in full-strength MS rooting medium compared to the half-strength MS rooting medium. Furthermore, IBA was more efficient in promoting root induction than IAA and NAA, resulting in a higher rate of root initiation (100%) at the concentration of 0.1 mg L<sup>-1</sup> IBA. Therefore, elongated shoots were rooted in MS medium supplemented with 0.1 mg L<sup>-1</sup> IBA for 3 weeks. Rooted plantlets were acclimatized successfully under ex vitro conditions.